Clinical Manifestations and Bacterial Genomic Analysis of Group A *Streptococcus* Strains That Cause Pediatric Toxic Shock Syndrome

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We report here 18 cases of pediatric group A streptococcal toxic shock syndrome, associated clinical findings, and bacterial molecular genetic characteristics discovered through whole-genome sequencing. This comparative whole-genome sequencing revealed unique gene content (*speK*) and polymorphisms (*dpiB*) in *emm87* group A *Streptococcus*, the relative contributions of which, in combination with the host response, in the development of streptococcal toxic shock syndrome remain to be elucidated.

Keywords. *emm* type; group A *Streptococcus*; invasive; shock; STSS.

Infections caused by group A *Streptococcus* (GAS) can vary greatly in their clinical presentation. Each year >600 million cases of pharyngitis and pyoderma caused by GAS occur worldwide [1, 2]. In comparison, invasive GAS infections such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS) are less common but result in substantially higher morbidity and mortality rates [2]. It remains unclear how GAS can cause benign infection in some people and invasive disease in others. Because of the propensity of GAS to result in outbreaks, molecular characterization of GAS strains that result in invasive disease is crucial for identifying potential clusters and preventing spread of the disease.

The bacterial genetic factors that contribute to the marked disease diversity in GAS strains have been the subject of

investigation for decades. STSS accounts for a small percentage (4%) of invasive GAS disease but is associated with a high mortality rate (~38%) [2]. A combination of bacterial (eg, streptococcal pyrogenic exotoxin [Spe]) and host (eg, specific human leukocyte antigen [HLA] haplotypes) factors can create a “perfect storm” in at least a subset of individuals, which leads to the high morbidity and mortality rates associated with STSS. In addition, some GAS *emm* types are associated more frequently with invasive disease (eg, *emm1*) and might be predisposed to genetic mutations that enhance their virulence and development of STSS [3].

Few studies have examined GAS strain characteristics associated with pediatric STSS. In this report, we present the clinical characteristics and bacterial strain features of patients with STSS at a large children's hospital between 2012 and 2016.

MATERIALS AND METHODS

STSS Case Definition and GAS Isolate *emm* Typing

We performed a single-center retrospective analysis of 341 cases of GAS disease (defined by the isolation of GAS from cultures of a sterile site and a nonsterile site) in pediatric patients aged ≤18 years who were admitted to Texas Children's Hospital between October 1, 2012, and June 30, 2016. STSS was determined by using the 2010 Centers for Disease Control and Prevention (CDC) criteria (see [Supplementary Methods](#)). The collection of GAS isolates and retrospective chart reviews were conducted under a protocol approved by the institutional review board at Baylor College of Medicine. No cases of STSS that tested positive for GAS by throat culture only were identified or included.

Whole-Genome Sequencing

Whole-genome sequencing (WGS) of GAS strains (see [Supplementary Table 1](#)) that resulted in STSS was performed as described previously [3] using an Illumina MiSeq instrument to generate 300-bp paired-end sequences. Raw reads were aligned to serotype-matched reference genomes for polymorphism identification, and de novo assembly was performed using CLC Genomics Workbench 11 and a custom pipeline.

Statistics

We used the Fisher exact test to compare the frequencies of mutations in STSS and non-STSS GAS isolates. A *P* value of <.05 was considered significant for all tests after Bonferroni correction.

RESULTS

Clinical Characteristics

Of the 341 patients, 18 met the CDC criteria for STSS in the first 72 hours of their hospitalization (see [Supplementary Figure 2](#)).

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The male/female ratio was 9:9, and the median age was 5.8 years (interquartile range, 2.2–9.4 years). The majority (83%) of the patients were younger than 10 years. Two-thirds of the STSS cases occurred during the winter and spring months (December through May), which coincides with the seasonal trends noted in national surveillance reports [2]. Fever was the most common presenting symptom (89%), followed by nonspecific gastrointestinal complaints (67%), respiratory distress (56%), and cellulitis (33%). A generalized macular rash was reported in 32% of the patients. All patients with STSS required escalation of care in the intensive care unit, and 2 of the patients died. Twelve (67%) patients required vasopressors, and 11 (61%) patients required mechanical respiratory ventilation; evidence of pneumonia or empyema was found in 8 of these 11 patients. Clindamycin was part of the initial antibiotic treatment for 50% of the patients. Penicillin was used in 78% of the patients after the identification of GAS. Intravenous immunoglobulin (IVIg) was not used for any of the patients during their hospital course. The majority of the organisms were isolated from blood (67%) or pleural fluid (22%). The remaining isolates were taken from joint fluid or muscle in those patients in whom evidence of septic arthritis and osteomyelitis was found (17%). Complete clinical characteristics, including key laboratory values, of patients with STSS are summarized in [Supplementary Table 2](#).

GAS Strain Characteristics

GAS isolates from each of the 18 patients with STSS were subjected to WGS. The most common *emm* type identified was *emm1* (*n* = 11), followed by *emm87* (*n* = 3), *emm12* (*n* = 2), *emm89* (*n* = 1), and *emm28* (*n* = 1). We next interrogated the WGS data to identify gene content or polymorphisms (single-nucleotide polymorphisms [SNPs] or insertions/deletions [indels]) that might be unique to our isolates and potentially contribute to an STSS phenotype. We used geographically diverse and recently sequenced invasive GAS strains from the CDC Active Bacterial Core Surveillance (ABCs) collection [4] as an *emm* type–matched non-STSS control group and identified no significant associations in gene content or polymorphisms between the STSS and non-STSS isolates in *emm1*, *emm12*, *emm28*, or *emm89* GAS. Given the previous observation of a cluster of severe invasive disease caused by *emm87* GAS in the same region [3], we examined the STSS-associated *emm87* isolates more closely. After combining these WGS data with data from the CDC ABCs (*n* = 26) [4], Canada (*n* = 12) [5], and our local surveillance (*n* = 37, including 27 previously sequenced isolates) [3], we compared a total of 75 *emm87* isolates (3 that caused STSS, 56 invasive disease, and 16 pharyngitis) in regard to their gene contents and polymorphisms. The phylogenetic relationship (ie, relatedness to the reference genome on the basis of SNPs) between *emm87* GAS isolates is shown in [Figure 1](#). We observed a significant association (*P* = .008, Fisher exact test) between STSS isolates and a nonsynonymous

mutation (which resulted in an arginine-to-cysteine amino-acid change at position 170 in the putative protein [R170C]) in the gene encoding the histidine kinase (*dpiB*) of the 2-component system (TCS) DpiBA ([Figure 1A](#)). In addition, 2 of the 3 STSS isolates contained the exotoxin gene *speK* ([Figure 1B](#)), but this finding was not statistically significant after we corrected for multiple comparisons.

DISCUSSION

We investigated STSS in a pediatric population at a large tertiary care hospital and identified 18 cases over a 44-month period. The majority of STSS cases identified in this study represent missed opportunities for optimal management. Current STSS treatment guidelines recommend the use of both clindamycin and a penicillin [6]. Moreover, evidence suggests that IVIg therapy can improve survival rates [7]. In our study, fewer than half of the patients received clindamycin, and none of them received IVIg. Given the high mortality rate associated with STSS, our observation emphasizes the importance of rapidly identifying STSS and administering appropriate combination therapy to treat it.

We observed *emm87* GAS as a cause of STSS in our population and identified potential *emm* type–specific genetic contributors to an STSS phenotype. The most common *emm* types that have correlated historically with shock and multiorgan damage have been *emm1*, *emm3*, *emm12*, *emm28*, and *emm89* [2]. Changes in gene content and mutations in regulators of GAS virulence also have been implicated in the development of invasive disease, including STSS. For example, mutations in TCS CovRS genes are known to contribute to invasive disease and STSS [8]. Bacterial TCSs consist of a sensor protein that, after recognition of an external stimulus, becomes activated via phosphorylation and subsequently activates a cognate response regulator that goes on to affect gene transcription. Thus, mutations that affect the activity of either the sensor or response regulator can have dramatic effects on bacterial gene regulation and phenotypic expression. We showed previously that a mutation in *covS* in *emm87* GAS contributed to a cluster of invasive disease within a single family [3]. Here, we extend our genetic analysis to include additional *emm87* GAS from the same region and include a geographically diverse *emm87* population using the CDC ABCs WGS data from 2015 [4]. Interesting is that we did not identify any mutations in *covRS* among the *emm87* population apart from the previously described familial cluster. Instead, the *emm87* STSS isolates contain an amino-acid-changing mutation (R170C) in the histidine kinase of a TCS (DpiBA). The DpiBA TCS (also referred to as MaeKR) regulates malate metabolism in GAS that has been shown also to influence virulence [9]. The effect of the DpiB amino-acid change in the *emm87* STSS strains is unknown, but it resides in the extracellular “sensing” domain and can alter activation.

Figure 1. Neighbor-joining (NJ) phylogenetic tree based on 760 biallelic single-nucleotide polymorphisms (SNPs) from 74 *emm87* GAS isolates from the United States and Canada. (A) NJ tree according to disease type (node color) and country of origin (node shape). Highlighted are strains associated with the previously reported cluster of *emm87* GAS disease in a single family [3] and strains with mutations significantly associated with streptococcal toxic shock syndrome (STSS). (B) NJ tree according to exotoxin gene content. Exotoxin gene abbreviations include streptococcal pyrogenic exotoxins (*speC*, *speG*, *speK*, *speL*, *speM*, *smeZ*, and *ssa* (S). *emm87* GAS isolates associated with STSS are highlighted. Abbreviation: REF, reference.

Streptococcal superantigens are critical virulence factors produced by multiple streptococci, including GAS (reviewed in reference [10]). Among *emm87* GAS strains, 78% (58 of 74) had identical exotoxin gene profiles (Figure 1B). Only 4 isolates harbored *speK*, of which 2 were STSS isolates. The *SpeK* exotoxin has been implicated in the emergence of streptococcal toxic shock-like syndrome in Japan [11]. In contrast, subtle differences in the host response to GAS infection can predispose a person to more severe disease. For example, Kotb et al [12] found that certain haplotypes of human leukocyte antigen class II increase the risk of severe systemic GAS infection, whereas others afford protection. The relative contributions of GAS virulence factors (eg, *dpiB* mutation and *speK* in *emm87* GAS) and the host response in the development of STSS remain to be elucidated.

Our study has several important limitations. First, patients with compensated shock were, by definition, excluded because they did not meet CDC criteria for age-specific hypotension. Similarly, cases of possible STSS (ie, GAS isolates were not available or the patients had abnormal laboratory findings that were below the cutoff laboratory criteria for multi-organ damage) were excluded. Therefore, our data most likely underestimate the true burden of STSS. Last, the current data are reflective only of cases during a short period of time, and the relative frequency of any specific strain causing STSS can change over time and according to region.

In summary, the rapid identification of severe disease caused by GAS is crucial given its associated high morbidity and

mortality rates. Furthermore, molecular characterization of GAS isolates and investigative studies of the host–pathogen interaction during STSS infection can lead to a better understanding of the disease process and improved medical interventions.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

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